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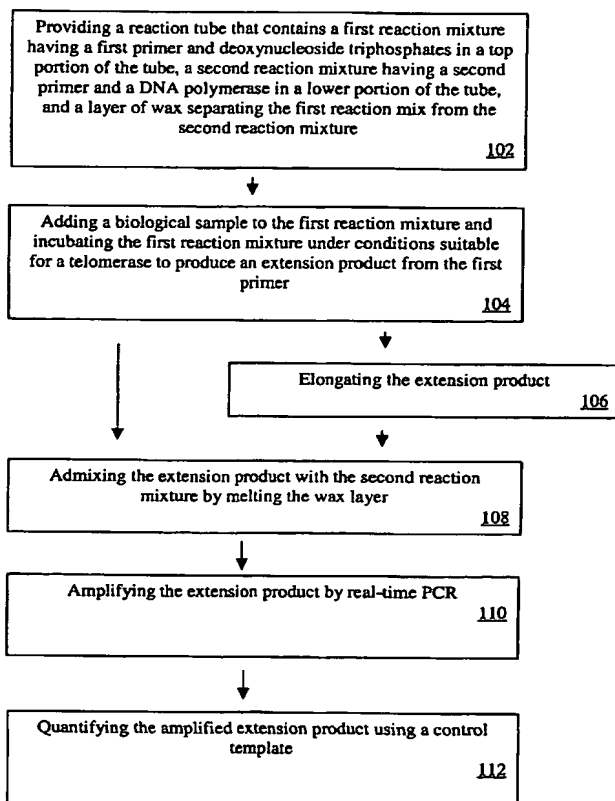
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[Continued on next page]

(54) Title: METHODS AND COMPOSITIONS FOR DETECTING TELOMERASE ACTIVITY



(57) Abstract: A method for determining telomerase activity using primer extension followed with real time PCR quantification is disclosed. The method of the present invention provides a rapid, sensitive and accurate measurement for telomerase activity in a biological sample. In one embodiment, the method includes the steps of: adding the biological sample to a reaction tube containing a first reaction mixture having a first primer and nucleoside triphosphates, a second reaction mixture having a second primer and a DNA polymerase, and a wax layer that separates the first reaction mixture from the second reaction mixture; incubating the biological sample with the first reaction mixture; admixing the extension product with the second reaction mixture; amplifying and quantifying the extension product using real-time PCR and a control template. In another embodiment, the detection method includes an *in situ* primer extension step that allows the production of the extension product within an intact cell. In this embodiment, the extension product can be preserved under appropriate conditions for an extended time before the completion of the quantification step.



(84) **Designated States (regional):** ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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— *without international search report and to be republished upon receipt of that report*

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## INTERNATIONAL SEARCH REPORT

Rec'd PCT/PRO

International Application No

16 MAY 2005

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A. CLASSIFICATION OF SUBJECT MATTER  
 IPC 7 C12Q1/68 C12Q1/48

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBASE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>ELMORE ET AL: "Real-time quantitative analysis of telomerase activity in breast tumor specimens using a highly specific and sensitive fluorescent-based assay" DIAGNOSTIC MOLECULAR PATHOLOGY, vol. 11, no. 3, September 2002 (2002-09), pages 177-85, XP008029655 cited in the application the whole document</p> <p>-----</p> <p>-/--</p>	<p>1-7, 15-18</p>

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

## \* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search

2 June 2004

Date of mailing of the international search report

10. 09. 2004

Name and mailing address of the ISA

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## INTERNATIONAL SEARCH REPORT

Intel International Application No

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## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>HOU M ET AL: "Real-time quantitative telomeric repeat amplification protocol assay for the detection of telomerase activity"            CLINICAL CHEMISTRY,            vol. 47, no. 3, 2001, pages 519-24,            XP002276809            cited in the application            the whole document</p> <p>-----</p>	1-7, 15-18
Y	<p>UEHARA H ET AL: "Detection of telomerase activity utilizing energy transfer primers: comparison with gel- and ELISA-based detection"            BIOTECHNIQUES,            vol. 26, no. 3, 1999, pages 552-58,            XP002276810            the whole document</p> <p>-----</p>	1-7, 15-18
Y	<p>US 5 891 639 A (HARLEY CALVIN BRUCE ET AL) 6 April 1999 (1999-04-06)            cited in the application            examples 2,4,5,8,10</p> <p>-----</p>	1-7, 15-18
Y	<p>US 6 221 584 B1 (EMRICH THOMAS ET AL) 24 April 2001 (2001-04-24)            cited in the application            column 3, line 62 - column 5, line 23            column 6, line 62 - column 7, line 19</p> <p>-----</p>	1-7, 15-18
Y	<p>US 6 391 554 B1 (WRIGHT WOODRING E ET AL) 21 May 2002 (2002-05-21)            figure 10</p> <p>-----</p>	1-7, 15-18
Y	<p>US 5 629 154 A (KIM NAM W ET AL) 13 May 1997 (1997-05-13)            figures 1B,1C</p> <p>-----</p>	1-7, 15-18
X	<p>SEIMIYA H ET AL: "Telomere shortening and growth inhibition of human cancer cells by novel synthetic telomerase inhibitors MST-312, MST-295 and MST-199"            MOLECULAR CANCER THERAPEUTICS, AMERICAN ASSOCIATION OF CANCER RESEARCH, US,            vol. 1, no. 9, 1 July 2002 (2002-07-01),            pages 657-665, XP002971865            ISSN: 1535-7163            the whole document</p> <p>-----</p>	20

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# INTERNATIONAL SEARCH REPORT

Intel

nal Application No

PCT/US 03/35919

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>BURGER A M ET AL: "Inhibition of Telomerase Activity by Cisplatin in Human Testicular Cancer Cells" EUROPEAN JOURNAL OF CANCER, PERGAMON PRESS, OXFORD, GB, vol. 33, no. 4, April 1997 (1997-04), pages 638-644, XP004282569 ISSN: 0959-8049 the whole document</p> <p style="text-align: center;">-----</p>	20

# INTERNATIONAL SEARCH REPORT

Inte

onal application No.  
PCT/US 03/35919**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:  
1-7, 15-18 and partially 20

**Remark on Protest**

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-7,15-18 and partially 20

A single-tube in vitro assay method for quantitative determination of telomerase activity in a cell sample using real-time PCR, and application of the method for monitoring the effectiveness of treatment of a patient.

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2. claims: 8-14, 19 and partially 20

An in vivo assay method for quantitative determination of telomerase activity in a cell involving real-time PCR, and application of the method for monitoring the effectiveness of treatment of a patient.

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 03/35919

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 5891639	A	06-04-1999	US 5863726 A	26-01-1999
			US 5837453 A	17-11-1998
			US 5629154 A	13-05-1997
			US 5989807 A	23-11-1999
			US 5830644 A	03-11-1998
			US 5645986 A	08-07-1997
			AU 6380896 A	15-05-1997
			JP 11507839 T	13-07-1999
			WO 9715687 A1	01-05-1997
			US 5804380 A	08-09-1998
			AT 193554 T	15-06-2000
			AU 682082 B2	18-09-1997
			AU 1209095 A	29-05-1995
			AU 6058298 A	04-06-1998
			CA 2173872 A1	18-05-1995
			DE 69424797 D1	06-07-2000
			DE 69424797 T2	28-12-2000
			DK 728207 T3	02-10-2000
			EP 0728207 A1	28-08-1996
			ES 2147602 T3	16-09-2000
			GR 3034249 T3	29-12-2000
			HK 1011384 A1	23-02-2001
			JP 11243998 A	14-09-1999
			JP 2875394 B2	31-03-1999
			JP 9502102 T	04-03-1997
			PT 728207 T	30-11-2000
			WO 9513381 A1	18-05-1995
			US 5648215 A	15-07-1997
			US 5639613 A	17-06-1997
			US 6391554 B1	21-05-2002
			US 5693474 A	02-12-1997
			AU 1178195 A	29-05-1995
			AU 1330795 A	29-05-1995
			WO 9513382 A1	18-05-1995
			US 5686306 A	11-11-1997
			WO 9513383 A1	18-05-1995
			US 2003190638 A1	09-10-2003
			US 6551774 B1	22-04-2003
US 6221584	B1	24-04-2001	DE 19644302 A1	05-06-1997
			AT 200108 T	15-04-2001
			AU 710326 B2	16-09-1999
			AU 2623197 A	19-06-1997
			CA 2237823 A1	05-06-1997
			CN 1202935 A	23-12-1998
			DE 59606675 D1	03-05-2001
			WO 9720069 A1	05-06-1997
			EP 0863999 A1	16-09-1998
			JP 11500015 T	06-01-1999
			JP 3289056 B2	04-06-2002
			NO 980699 A	19-02-1998
			NZ 322938 A	25-02-1999
US 6391554	B1	21-05-2002	US 5989807 A	23-11-1999
			US 5830644 A	03-11-1998
			US 5645986 A	08-07-1997
			US 5695932 A	09-12-1997
			US 5489508 A	06-02-1996



# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 03/35919

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 6391554	B1	AT 193554 T	15-06-2000
		AU 682082 B2	18-09-1997
		AU 1209095 A	29-05-1995
		AU 6058298 A	04-06-1998
		CA 2173872 A1	18-05-1995
		DE 69424797 D1	06-07-2000
		DE 69424797 T2	28-12-2000
		DK 728207 T3	02-10-2000
		EP 0728207 A1	28-08-1996
		ES 2147602 T3	16-09-2000
		GR 3034249 T3	29-12-2000
		HK 1011384 A1	23-02-2001
		JP 11243998 A	14-09-1999
		JP 2875394 B2	31-03-1999
		JP 9502102 T	04-03-1997
		PT 728207 T	30-11-2000
		WO 9513381 A1	18-05-1995
		US 5891639 A	06-04-1999
		US 5629154 A	13-05-1997
		US 5648215 A	15-07-1997
		US 5639613 A	17-06-1997
		US 5837453 A	17-11-1998
		US 5693474 A	02-12-1997
		US 5863726 A	26-01-1999
		US 5804380 A	08-09-1998
		AU 1178195 A	29-05-1995
		AU 1330795 A	29-05-1995
		WO 9513382 A1	18-05-1995
		US 5686306 A	11-11-1997
		WO 9513383 A1	18-05-1995
		US 2003190638 A1	09-10-2003
		US 6551774 B1	22-04-2003
		US 2002127634 A1	12-09-2002
		US 6368789 B1	09-04-2002
		AU 688262 B2	12-03-1998
		AU 4374093 A	13-12-1993
		AU 7183698 A	20-08-1998
		AU 8949598 A	07-01-1999
		AU 8949698 A	14-01-1999
		CA 2135648 A1	25-11-1993
		CA 2245461 A1	25-11-1993
		CA 2245462 A1	25-11-1993
		EP 0642591 A1	15-03-1995
		JP 11123100 A	11-05-1999
		JP 11127874 A	18-05-1999
US 5629154	A	US 5989807 A	23-11-1999
		US 5830644 A	03-11-1998
		US 5645986 A	08-07-1997
		AT 193554 T	15-06-2000
		AU 682082 B2	18-09-1997
		AU 1209095 A	29-05-1995
		AU 6058298 A	04-06-1998
		CA 2173872 A1	18-05-1995
		DE 69424797 D1	06-07-2000
		DE 69424797 T2	28-12-2000
		DK 728207 T3	02-10-2000
		EP 0728207 A1	28-08-1996

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 03/35919

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5629154	A	ES 2147602 T3	16-09-2000
		GR 3034249 T3	29-12-2000
		HK 1011384 A1	23-02-2001
		JP 11243998 A	14-09-1999
		JP 2875394 B2	31-03-1999
		JP 9502102 T	04-03-1997
		PT 728207 T	30-11-2000
		WO 9513381 A1	18-05-1995
		US 5891639 A	06-04-1999
		US 5837453 A	17-11-1998
		US 5863726 A	26-01-1999
		US 5804380 A	08-09-1998
		US 5648215 A	15-07-1997
		US 5639613 A	17-06-1997
		US 6391554 B1	21-05-2002
		US 5693474 A	02-12-1997
		AU 1178195 A	29-05-1995
		AU 1330795 A	29-05-1995
		WO 9513382 A1	18-05-1995
		US 5686306 A	11-11-1997
		WO 9513383 A1	18-05-1995
		US 2003190638 A1	09-10-2003
		US 6551774 B1	22-04-2003